

CLAIMS

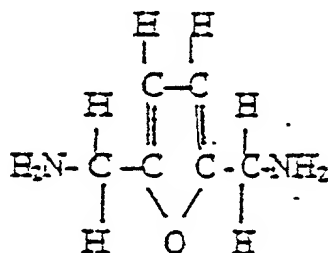
1. In a method of cell culture wherein cells are cultured on microcarrier beadlets the improvement comprising growing the cells on dextran microcarrier beadlets and thereafter separating the cells from the microcarrier beadlets by digestion with dextranase.
2. The method of claim 1 wherein the cells are a member of the group consisting of chondrocytes, osteocytes, osteoblasts, monocytes, chondrogenic cells, pluripotential cells, mucosal cells and fibroblasts.
3. The method of claim 2 wherein the cells are chondrocytes or chondrogenic cells.
4. The method of claim 3 wherein after separating the chondrocytes or chondrogenic cells from the microcarrier beadlets the cells are plated on a scaffold or template for growth thereon.
5. The method of claim 4 wherein after the cells are plated on a scaffold or template for growth thereon, the scaffold or template is surgically implanted into the cartilage tissue.
6. The method of claim 1 wherein after separating the cells from the microcarrier beadlets the cells are injected into a patient in need of said cells.

7. A crosslinked polymer prepared by crosslinking a polysaccharide with a polyamine said polymer to be used for cell culture and preparing scaffolds or templates for tissue replacement.
8. The crosslinked polymer of claim 7 wherein the polysaccharide is selected from the group consisting of arabinogalactan and oxidized arabinogalactan; and the crosslinking agent is glutamic acid and lysine, albumin, gelatin or lysine.
9. The crosslinked polymer of claim 7 wherein the polysaccharide is selected from the group consisting of dextran, cellulose, arabinogalactan, pollulan and amylose.
10. In a method of cell culture wherein cells are cultured on microcarrier beadlets the improvement comprising growing the cells on a polyamine cross-linked polysaccharide polymer of claim 7 and thereafter separating the cells from the microcarrier beadlets by digestion with an enzyme compatible with the polysaccharide.
11. The method of claim 10 wherein the cells are a member of the group consisting of chondrocytes, chondrogenic cells, osteoblasts, monocytes, pluripotent cells, mucosal cells and fibroblasts.
12. The method of claim 11 wherein the cells are chondrocytes or chondrogenic cells.

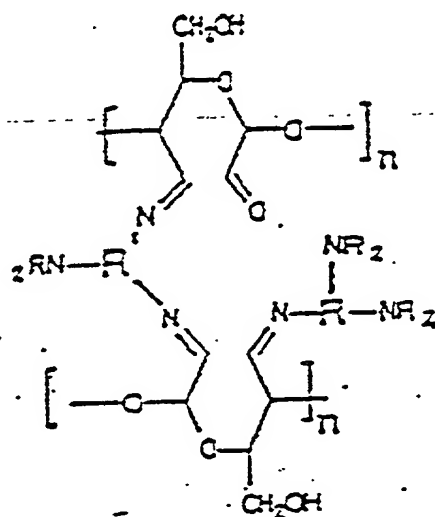
- [illegible]

16. The beadlet for cell culture or the scaffold used for cartilage repair of claim 15 wherein the polysaccharide used to prepare the crosslinked polysaccharide derivative

is a member selected from the group consisting of dextran, arabinogalactan, pollulan, cellulose and amylose; with the polysaccharide derivative being crosslinked by a polyamine compound selected from the group consisting of lysine, ethylenediamine, alkylenediamine, phenylenediamine, xylenediamine, polyethylenimine, gelatin, albumin, fibrinogen and

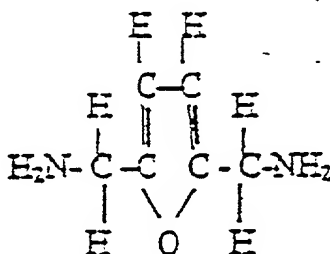


17. A beadlet for cell culture or a scaffold used for cartilage repair comprising a crosslinked polysaccharide derivative of the formula



n is an integer of between 2 and 10,000 and R=H, alkyl or alkylene, and the polysaccharide derivative being crosslinked by a polyamine compound.

18. The beadlet for cell culture, or the scaffold used for cartilage repair of claim 17 wherein the polysaccharide used to prepare the crosslinked polysaccharide derivative is a member selected from the group consisting of dextran, arabinogalactan, pollulan, cellulose and amylose; with the polysaccharide derivative being crosslinked by a polyamine compound selected from the group consisting of lysine, ethylenediamine, alkylenediamine, phenylenediamine, xylenediamine, polyethylenimine, gelatin, albumin, fibrinogen and



19. The method for the surgical repair of cartilage wherein the crosslinked polysaccharide derivative of claim 15 in the form of a scaffold containing chondrocytes thereon is surgically implanted into the cartilage tissue of a patient.
20. The method for the surgical repair of cartilage wherein the crosslinked polysaccharide derivative of claim 17 in the form of a scaffold containing chondrocytes thereon is surgically implanted into the cartilage tissue of a patient.
21. A scaffold or template for cartilage repair comprising a chitosan scaffold or template with chondrocytes incorporated thereon to produce a scaffold or template for cartilage repair having viable chondrocytes.

22. A method for the surgical repair of cartilage tissue wherein the chitosan scaffold or template with chondrocytes of claim 21 is surgically incorporated into the cartilage tissue of a patient.
23. A scaffold or template for cartilage repair comprising a member of the group consisting of chitosan and a crosslinked polysaccharide prepared by crosslinking said polysaccharide with a polyamine, said member being shaped in the form of a scaffold or template and having plated and growing thereon chondrocytes.
24. In a surgical procedure for surgically implanting chondrocytes into cartilage tissue wherein the scaffold or template having plated and growing thereon the chondrocytes of claim 23 is surgically implanted into the cartilage for cartilage repair.
25. In the surgical procedure of claim 24 wherein the scaffold or template having plated and growing thereon chondrocytes is surgically implanted into cartilage of the knee.
26. In the surgical procedure of claim 24 wherein the scaffold or template having plated and growing thereon chondrocytes is surgically implanted into cartilage of the head or neck area.
27. In the surgical procedure of claim 24 wherein the scaffold or template having plated and growing thereon chondrocytes is surgically implanted into the knee of a horse.

28. In a surgical method for surgically implanting chondrocytes into cartilage tissue comprising the steps of:
- 1) culturing chondrocytes on dextran microcarrier beadlets and thereafter separating the chondrocytes from the microcarrier beadlets by digestion with dextranase,
 - 2) plating and growing said separated chondrocytes on a scaffold or template manufactured from a crosslinked polysaccharide prepared by crosslinking said polysaccharide with a polyamine or alternatively plating and growing said separated chondrocytes on a scaffold or template comprising chitosan and then
 - 3) surgically implanting into cartilage tissue said scaffold or template having chondrocytes plated and grown thereon, to thereby repair cartilage tissue in a patient requiring cartilage repair.
29. In combination chitosan and a member of the group consisting of osteoblasts and chondrocytes wherein the chitosan serves as a substrate for the growth of said osteoblasts or chondrocytes.
30. The combination of claim 29 wherein the chitosan is in the form of a scaffold to be used in transplantation surgery.

31. A method of culturing and obtaining increased levels of collagen type II chondrocytes comprising culturing the chondrocytes at a low oxygen tension.
32. The method of claim 31 wherein the oxygen tension is about 5%.
33. In a surgical procedure wherein chondrocytes are taken from a patient rapidly multiplied and transplanted into said patient comprising the steps of:
- 1) obtaining cartilage tissue from a patient,
 - 2) treating the cartilage to obtain chondrocytes therefrom,
 - 3) culturing the chondrocytes to obtain an adequate sample of chondrocytes,
 - 4) plating and growing the chondrocytes onto a chitosan scaffold, and
 - 5) surgically implanting the chondrocytes bearing chitosan scaffold into said patient, wherein the entire surgical procedure is carried out expeditiously with good quality chondrocytes being returned to the patient and the scaffold being reabsorbed leaving in place viable chondrocytes.
34. A method for preparing a scaffold for tissue repair comprising culturing chondrocytes onto a chitosan scaffold wherein the culturing takes place in the presence of integrins.

35. The method of preparing a scaffold for tissue repair comprising culturing chondrocytes onto a chitosan scaffold wherein the culturing takes place at a low oxygen tension under controlled conditions in an automated spin culture.

LEONARD BLOOM & ASSOCIATES, LLC
401 Washington Avenue, Suite 905
Towson, Maryland 21204
(410) 337-2295
SR/mjr; 3-4-99

A:\97276-2-11.doc